EFFECT OF SYNTHETIC PYRETHROIDS ON THE DIGESTIVE TRACT OF SOME INSECT PESTS OF AGRICULTURAL CROPS

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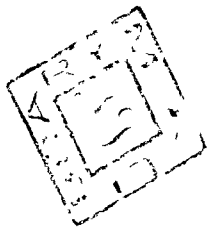

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This is to certify that the dissertation for M.Phil. degree in Agriculture of the Aligarh Muslim University, Aligarh has been completed by **Mr. ISRAR AHMAD ANSARI**, under my supervision. It is original in nature and I have permitted the candidate to submit it in partial requirements for the award of the degree.

(**HUMAYUN MURAD**)

Supervisor

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ABBREVIATIONS

ALnt	...	...	Anterior intestine
BMb	...	...	Basement membrane
Cel	...	...	Columnar cell
Cmcl	...	...	Circular muscle
Cr	...	...	Crop
Ct	...	...	Connective tissue
Cyct	...	...	Cytoplasm
Epcl	...	...	Epithelial cell
G cl	...	...	Goblet cell
IPM	...	...	Integrated pest management
Lmcl	...	...	Longitudinal muscle
Lm	...	...	Lumen
Mag	...	...	Magnified
Mal	...	...	Malpighian tubule
Mesen	...	...	Mesenteron
Nu	...	...	Nucleus

Ni	...	...	Nidus
Oe	...	...	Oesophagus
PLnt	...	...	Posterior intestine
Proct	...	...	Practodaeum
PMl	...	...	Peritrophic lamella
PMb	...	...	Peritrophic membrane
RH	...	...	Relative Humidity
Rect	...	...	Rectum
Rgc	...	...	Regenerative cell
Rgn	...	...	Regenerative nuclei
Sb	...	...	Striated border
Stom	...	...	Stomodaeum
Tc	...	...	Trachea
Vc	...	...	Vacuole

GENERAL INTRODUCTION

GENERAL INTRODUCTION

India is predominantly agricultural country with nearly 70 percent of its total working population engaged in Agriculture. It account for more than half of the total national income and it is therefore, the mainstary of Indian economy. It is quite clear that the development of Agriculture has much to do with the economic welfare of our country. Our country having a varied climatic conditions is in unique position to grow almost every possible crop and occupies an outstanding position in the world with respect ot several agricultural products. In India annual crop losses are estimated at Rs. 6,000 to 7,000 crores, of these aproximately 26 percent loss in caused by insects and rodents while weeds, disease, birds and nematods etc. acocunt for the rest. More than 70 percent of the total pesticides used are insecticides. 12-15 percent fungicides and abount 4-5 percent are weedicides. The emphasis in taking the losses arising from pest needs an orientation on the basis of constraints. (**Muthuranan, 1995**).

Like every other organism an insects is inseparable part of its environment. Insect and its environment are mutually interactive and adjustable. Nature has provided insects with different types of mouthparts by which they may cause damagge to man, animals and crops. "An insect whose population increases to the extent that it starts causing annoyance in conveneince or injury to man, his animals, plants material possessing will be called a pest". Similarly The Oxford advanced leaneris Dictionary of current English difines pest as a troublesome or destructive thing, animal etc, where as (William, 1947) states "an insect pest is any insect in the wrong place from a human point of view, just as a rose blush is a weed when it is growing in a cabbage patch". At this junction we can say, an insect whose population increases to an extent as to cause economic losses to crops or a nuisance and health hazard to man and his livestock will be declared a pest.

An ordinary layman only knowns about chemicals to check pest population. But recent trend is the use of Integrated pest management (I.P.M.). The two main factors lead to the origin and evolution of the concept of IPM are, hazards of insecticides and economy of pest

control, in view of the high cost of insecticides of the total 90,000 tonnes of annual pesticides usage only 63 percent is meant for agricultural purpose (**Muthuraman, 1995**). Nearly 600 corre rupees agro pesticides market is concentrated in Andhra Pradesh, Gujrat, Karnataka, Punjab and Uttar Pradesh.

The applicaiton of pesticides according to the economic threshold level in one of the recommandation in IPM. The economic threshold level of a pest is influenced by following major factor;

(i) Price factor

(ii) Varietal behaviour

(iii) Age of the crop

(iv) Influence of other pests.

Since the plants need to be protected against pests and insects the use of pesticides and insecticides has helped in increasing agricultural production. Insecticides can be classified in three ways;

(i) According to their mode of action

(ii) According to their mode of entry in the body

(iii) According to their chemical nature

The use of any insecticide depend chiefly on the insecticidal property of its active agent. Some of the insecticides are phytotoxic therefore can not be used indiscriminataly. Origanochlorines are more persistant as compared to oroganophosphates or carbonates. The recent breakthrough of Synthetic pyrethroids viz, Permethrin as Hilthrin, Cypermethrin as Ripcard, Deltamethrin as Decis, fenvalerate as sumicidin and Relothrin has widened the adoptation of IInd generation insecticides. Very little of synthetic pyrethroids exhibit high activity against insects low mammalian toxicity, greatly increased stability effectiveness at very low dosages rapid action and degradation to innocuous residues. On the other hand the remaining are plant originated insecticides safe to the environment biodegradable and non-toxic to human beings.

Several evils are associated with the widespred use of synthetic pesticides in agriculture. In the long run, the multifarious harmful effects of chemical pesticides outweigh their benefits. Synthetic oroganic pyrethroids have some advantage of which their effectiveness is one step forward. Synthetic pyrethroids are less biodegradable while the plant originated insecticides are

highly biodegrable, they do not pose any threat to the environment and its components. The problem of residue insecticides on food and fodder crops has emphasized the necesity of using such insecticides which are less toxic to mammals, easily degradable into non-toxic components in a short time. Insecticide of Synthetic pyrethroids can be usefully employed to control a number of insect pests of agricultural crops.

Synthetic pyrethroids have extremely high contact activity and are particularly effective against Lepidopterous larvae (King and Saunder's, 1984). They have a low persitance and hence require appropriately timed applicaiton and effective at very low doses. Majority of the pyrethroids have a very low mammalian toxicity (Elliott. et al., 1978). The pyrethroids are the most recent major development for the agrochemcial industry, now accounting for about one third of world insecticides. The development of new leads for insecticides is largely dependent on state funded agencies.

Pyrethroids constitute a new generation of highly active synthetic insecticides. They are derived in a group of insecticidal esterns, the pyretherin which can be

extracted from the flower heads of certain **Chrysanthemum** species. The pyretherins and early pyrethroids are suseptible to rapid degratation in environment. After the replacement of photosensitive groups in these compounds a great range of stable insecticides has been developed, which are generally more effective against a bread spectrum of economically important pests than the most potent organochlorine, organophosphate and carbonate insecticides. The high insecticidal potency is combined with a low oral toxicity to mammals an other nontarget species of aquatic organism excluded. Pyrethroids in contrast to the much persitant chlorinated hydrocarbon insecticides do not accumulate in the ecophere. There favourable toxicological and ecological properties instigate the wide spread application of pyrethroids in the control of diseases vectors, ectoparasites and pests **infesting** important agricultural crops. Around 1980 photostable pyrethroid had alrady grained 30% of world market of insecticides (**Naumann, 1981**).

The promising features of pyrethroids for pest control have further stimulated the detailed examination of their mechanism of action. Although the

neurotoxic action of pyrethrins was already recognised about a century ago, only recently the highly specific neurotoxic action of pyrethroids on the cellulos and subcellular level begun to be revealed with the use of advanced electrophysiological and biochemical techniques. It has been determined that the sodium channel protein in the nerve membrane is one of the primary target of pyrethroids on the nervous system is to induce repetitive activity. Trains of nerve inpluses dominate nervous activity and replace to normal pattern of single impulses. The dischages completely disturb the proper functioning of the entire nervous system. Sense organs, nerve endings and neurosecretary organs appear to be specially affected resulting in hyperxicitations, inco-ordination and 2paralysis. Excessive release of neurohormones will lead to a severe imbalance of the various bodily function, other membrance proteins, such as ATPases, may also be directly affected by pyrethroids. Differences in toxicokineties and in the metabolic degradation of mammals to these compounds and are also responsible for some cases of arthropod resistnace which have surfaces recently. A nerve insensitivity factor probably accounts for cross -

resistnace of DDT insensitive insect to pyrethroids. The highly specific action of pyrethroids has further prompted ther use as tools in study of subcellular processes in excitable membrances (Ruigt. 1985).

The *Pericallia ricini* Fabr. (Lepidoptera: Arctiidae) is mainly a pest on *Ricinus communis* L. (castor). P.ricini adult is grey-brown, with series of dark spots with light margius. The hairy eaterpillar is a pest of pulses, vegetables, castor, Fodder crops and Cucurbitacea etc. and is generally spread over whole of India. The female lays eggs underside of the leaves and paper stipes. 3-4 days after its emergence. The larva grows feeding on the surface tissue of the leaves in the early stages and on whole leaf tissue in the later instar and becomes full grown in 25-30 days undergoing seven instars. Damage is done only by caterpillar. They feed gregariously in earlier stages but later on become voracious feeders of foliage. It is important to study the physiology of the gut of a pest due to future control practices to be adopted. However, in an experimental study morphological and histological changes during feeding has to be studied. The agricultural importance of pest necessiates the study of histopathology

and physiology which may be helpful in the control of insecct pest. On account of being highly evolved Lepidopterous insect *P. recini* makes the study all the more essential.

Although the synthetic pyrothroids are mainly Neurotoxic in action they are have been showing to be effective add the celular and subcellular cells. The present study has been undertaken with the idea as to whether in field conditions such Pyrethroids when enter the alimentary canal have any adverce effect on the gut lining. It is also presumed that insects when they feed on the foliage where synthetic pyrethroids have been applied may also affect the mouth part there by acting as antifeedent. The present study has been undertaken in three parts; Part one - general anatomy of alimentary canal, Part Two histology of midgut and Part Three histopathlogy of midgut 4th instar larvae of *P. ricini*.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Several authors have studied the effects of insecticides on midgut epithelium of different insect viz: Pilat (1935), Woke (1940), Salkeld (1951), Chadbourne and Rainwater (1953), Mukherji and Hardas (1954), Soliman and Soliman (1958), Sharma (1966), Lakshmanlal et al. (1970), Rizvi (1977), Sabesom and Ramlingam (1978), Zutshi and Saxena (1989) and Singh (1990). Insecticides effect the normal functions of specific cells and tissue of insects body and make the survival of insect difficult. A knowledge of insecticides in the insect body is, therefore, a pre-requisite for divising and adopting control mesures . It is also useful for selection, improvement and application of insecticides.

The pathological effects of various insecticides on different organs of insect have been reported by various entomalogists including Parfentjev (1929) the pioneer worker on the digestive tract.

Later different workers investigated effect of various insecticides on the midgut epithlium of different insects the recent amongs them include **Blajjewska** (1964) **Topozzada, et al;** (1968), **Shukla et al;** (1977) and **Sabesan** and **Ranlingam** (1978).

The Lepidopterous insects, first histopathological work was done by **Pilat** (1935) who made a histological picture of midgut of *Vanessa urticae* L. (Tortoise shell butterfly), *Porthetria disper* L. (Gypsy moth) and *Pieris brassicae* (Cabbage butterfly) after they had been fed on leaves poisoned with Sodium Silicofluoride, Sodium fluoride Sodium and Calcium arsenate and paris green. In case of *V. urticae* treated with sodium fluoride, Sodium Silicafluoride paris green and calcium arsenate, the anterior part of midgut was entirely deprived of midgut epithlium and the wall of midgut was represented by only a connective tissue membrane and muscularis layer the damage was also marked towards the posterior end where the epithlial cells were found to be intact and apparently normal.

In *Porthetria dispar* L. poisoned with sodium silicofluoride, there was no significant histological

changes in the midgut epithalium of larva. The larvae of *Pieris brassicae* poisnoed with sodium arsenate and sodium silicofluoride showed certain histological changes. By sodium silicofluoride the nuclei of cells showed a more compact structure the Sodium arsenate poisoned larvae had a greater resemblence to the normal one. Such histopathological affects were generally observed in those caterpillars which showed violent symptoms of poisoning before dissection.

Woke (1940), Observed the effect of lethal doses of certain arsenicals (Lead aresenate; paris green, calcium arsenate, arsenic trioxide), barium fluosilicate, sodium fluoride, sodium fluoaluminate, phenothiozine and rolenone on the midgut wall of the sixth instar larvae of army worm *Prodenia eridania* cram. It was recorded that ingestion of higher doses of arsenicals was followed by disintegration of the midgut epithelial cells and damage to the midgut muscle fibres. Such damage was more profound after long intervals following the intake of these arsenicals. By the ingestion of Sodium fluoride these was disintegration of the substance of cytoplasm and nuclei of the epithelial cells. Similar marked distingration followed

the ingestion of sodium fluoialuminate. The sequence of pathological changes in the midgut epithlium involved vacualization in the cytoplasm, disappearance of striated border, disorganization in the epithelium. The ingestion of approximately 1.0mg of arsenic trichloride (oxide) did not cause any appreciable damage. However, higher doses caused severe damage to midgut epithleium 0.5 mg to 1.0 mg of barium fluosilicate caused an adverse effect on the midgut epithelium of the larvae when administered orally. But ingestion of sodium fluoxide between 2.0 mg to 5.0 mg was found enough to cause disintegration of the epithelial cytoplasm and nuclei similarly, disorganization appeared in the epithlial cells by the intake of 1.0 mg. of sodium fluoaluminate phenthiazine in a dose of 1.0mg was as ineffective as 50 mg of relenone to the midgut epithelium.

Richard and Cutkomp (1945) Could not observe any histopathological changes in the midgut epithlium of *Periplaneta americana* by poisoning with DDT. furhter Day and Powning (1949) observed breakdown of epithelial cells by feeding arsenic compounds to Blatella. They also found that Chlordance produces loss of striated border, Separation of epithelial cells. Cytolysis and that BHC Caused

vacualization in the cytoplasm of the epithelial cells.

In Hymenoptera the histopathology of midgut was first studied by **Salkeld** (1950) by oral administration of graded doses of lead arsenate, DDT and parathion. These insecticides caused histological abnormalities, mostly confined to the middle region of the ventriculus. The arsenic poisoned bees were found to be lighter in colour than the normal bees and 90% contained a greyish plug like mass near their posterior end. There was severe vaccualization and degeneration of the epithelial cells.

The midgut of 50-60% of DDT poisoned bees contained a large transparent gas bubble due to which the epithelial lining was stretched and there was increased proliferation, vacuolization as well as hypersecretion from the epithelial cells. The microscopic and macroscopic appearance of the midgut of parathion poisoned bees did not show any change in comparison to the normal bee. Later, **Salkeld** (1951) made a detailed study on the effect of acid lead arsenate (PbH AsO_4) parathion, pure isomer of DDT 1,1,1, trichlor - 2,2 his (P-Chlorophenoly) ethane 50% DDT wettable powder Niatox brand as stomach poisons on the midgut of the honey bee. There was hyper activity in DDT and

parathio poinoned bee due to the action of these insecticides as nerve poisons. In the arsenic poisoned bees these was marked vacuolization, defoliation and degeneration of the epithalial cells of midguts. No apparent histological changes was observed in the parathion poisoned bees which had large gas bubble in the ventriculi and their epithlium was streched and increased.

Histopathological studies of the midgut of the larvae of *Helithothis armigera* was made by **Chadbourne** and **Rainwater** (1953) to determine the effect of calcium arsenate, DDT and Dieldrin. Calcium while DDT and dieldrin were toxically applied to the first pair of abdominal appendages. Tissues showed marked degeneration when treated with calcium arsenate, almost the entire epithelium was completely disintegrated and finally sloughted off into the midgut lumen. DDT treated larvae failed to show any significant histological changes in the midgut epithelium. Dieldrin treated larvae showed marked degeneration and other abnormalities in the midgut epithlium, Dieldrin affected more severely than calcium arsenate and midgut epithelium was degenerated.

Mukerji and Haridas (1954) reported some histopathological changes in the third instar nymphs of *Schistocerca gregaria* by the ingestion of BHC, Parathion, Chlordane and Dieldrin. These insecticides caused great disintgradation and exfoliation of midgut epithelium and BHC also disintegrated the peritrophic membrane and their toxic action was found to be more serve in the posterior region of the midgut than in the anterior part. Epithelial cells detached from the basement membrane nuclei enlarged, nuclear membrane become thin and the nuclear chromatin clumped.

A study on the histopathology of the midgut of fully grown larvae of *Prodenia litura* was made by saliman and Soliman (1958) by dusting parathion DDT Toxaphone one cotton dust. The larvae treated with parathion showed most profound histological changes in the midgut epithalium which completely detahced itself from the basement membrane. Further, turgidity and striations of the musculosa was lost and empty spaces appear in the fibres. In the DDT treated larvae epithelium exfoliated from the basement membrane in the form of large sheats in which some of the cells retained their typical histological characters. The peritrophic membrane was completely destroyed . In the toxophine

poisoned larvae, great distingration and destruction of the epithlium of the midgut was reported. The destruction was accompanied by oxfoliation of epithelial cells from the basement membrane, Nuclear chromation formed a compact mass which was broken into intensely coloured separate lumps of different size and complete obliteration of the structure of these nuclei was observed Peritrophic emmbrane left no traces.

The feeding of sodium aresenite, lead arsenate, sodium fluosilicate, zinc phosphide , chlordane and BHC to *Leogryllus bimaculatus* sauss, *Periplaneta americana* L. and *Gryllodes sigillatus* walk caused great disintegration of the epithelial cells of the midgut **Srivastava** (1959). It was accompanied by the obliteration of the midgut epithelium and complete destruction of the brush border. The inner margin of the cells had large extrusions of irregular mass of cytoplasm. Later, exfoliation of the epithelial cells occured and finally entire epithelium seprated from the muscularis layer Generally nuclei remained unaffected, but with the prosphide the chromatin granules appeard to arrange along the nuclear membrane of the epithelial cells of midgut.

The larvae of cabbage butterfly, *Pieris brassicae*, **Blazeiwska** (1964) were fed on cabbage leaves smeared on the lower surface with on 0.3% aquous suspension of Tritox dust (a mixture of DDT, BHC, Methyoxy DDT) These chemicals caused destruction of the midgut epithelial cells. Similar histogical changes were recorded when the larvae released on the dust of these chemicals which acted as contact poisons.

Sharma (1966), reported the effect of topical application of allenthrin, dieldrin, nuvan and parathion on *Pecilocerus pictus*. The histopathological changes were serve in the form of cavuolization and degeneration of midgut epithelium but these was no exfoliation. Destruction in the foregut was more pronounced than that of the midgut epithelium.

Toppozada et al; (1968) observed the effecct of feeding and tropical applciation of the carbaryl, parathion, DDT and endrin on the larvae of *Spotoptera littoralis* and they reported considerable cytopathological changes in the midgut epithelium. It was found that in the midgut, the ingestion of these insecticides caused rapid and maximum pathological effects as compared with the result of

topical applications. It was further noted that several cytological changes progressed in the midgut, comprising elongation of the epithelial cells, vacualization, fading of cell boundries chromatin clumping, lyris, shedding of cytoplasm and degeration of cells. There is a definite synchromatization between the external toxicity and the pathological effects on the midgut of *Spodoptera littoralis*.

Histopathalogical effects of endrin, Dipterex seven and Lebacid on the cuticle midgut, malpighian tubules, muscles and fat body of the larvae of *Earis insulana* Boid, were observed by **Massanuss** (1968). These insecticides caused vacuolization in the epithelial cells detachment from the basement membrane and finally disintegration of the cells was reported.

Lakshmanlal et al; (1970) reported histopathological effect of the injection of endosulfan, diazinon and dichlorvos in the second pair of abdominal prolegs of tobacco caterpillar, *Spodptera litura*. There was general destruction and disintegration in the midgut epithelum accompanied by the shedding of cytoplasm, disappearance of cell boundaries, shrinkage of the

epithelial cells with vacoules degeneration and chromation clumping of the nuclei of the gut epithelium. Moreover, effect of chlorinated hydrocarbons (DDT lindane, endrin and aldrin) and an arganophosphorus compound (Parathion) as well as that of stomach poisons (sodium silicofluoride and copperr acetomate arsenate) were studied on the nymphal stages and adult of *Hieroglyphaus nigrorepletus* (Rizvi, 1970; Rizvi and Khan, 1973). Different concentratioon of these insecticides were given orally. The histopathological changes which occured in the midgut and caeca of this species were in the form of discharge of cytoplosmic granules, globules and vesicles with nuclei, nipping offenner margin of the epithelium. in the most severe cases of damage dissolution and degeneration occured. The degeneration of nuclei of the digestive and regenerative cells was manifestive and regenerative cells was manifested by the gradual fusion of their chromatin granules which finally formed a charge amorphus mass. The completely degenerated nuclei had reputured membrane and their amorphus mass were extruded. The effectiveness of each insecticide depended upon its concentration and internal following its feeding. Complete degeneration and obliteration of midgut

epithelium occured in all stages of *H. nigrorepletus* with respect ot strong doses.

In a study by **Sabesan & Ramalingan** (1970) on the efects of the insecticide endosulfan on the midgut epithelium and its secretary activity in the adult *Odontopus varicorn*. Signs of degeneration, shrinkage & indistinct cell boundaries of midgut epithelium were found in endosulfan treated condition The epithelium of this midgut region stood separated from the muscular layer. Cytoplasm of the midgut epithelial cells also should spasse distribution and cytoplasmic granules while nuclei of these cells became pyenotic and indicated sings of chromatic clumping.

Zutshi & Saxena (1989) have studied the effect of pyrethrum on the fore and midgut of *Grylledes Singillatus* worker administered by feeding different concentraiotn of pyrethrum 1% and 2% to the instar revealed vasualisation [illegible] spaces, eccelerated secretion, increase in the regeneration activity of cells, rejection of alder cells and pynotic epithelial cells as the general histopathological damage.

The histopathological effects of LC 50 values of carboryl, endrin, lead arsanete and ethyl parathian were

studied on the alimentary canal of adult female of *chrologonus trachypeterus* by **Singh** (1990). The insecticides were given orally and it was found that ethyl parathin badly damaged oesophagus and midgut while epithelial cells were not desentegrated endrin carboyel and assandle bodly damaged the midgut the epithelium found to completely disintegrated. None of the insecticide damaged the hindgut. The above resume indicates that these information on histopathologically effects on different tissues of insect is fragmentary in comparison to the mass population of destruction insects and ever increasing number of insecticides which have been put in use of control these pests. Therefore in the present work a modern synthetic Pyrethroids compound has been selected which has been recommanded as a board spectrum insecticides to control different types of insect pests of agricultural crops. The active ingredient of this insecticide has been topically used on the 3rd instar larvae of *Pericallia ricini* F. (Lopidoptera, Arctiidae) in order to observe the pathological effect on the histological structures of midgut. Further the application of Deltamethrin and Relothrin of the larvae of *P. ricini* ,F will add to our

histopathological knowledge on the above mentioned tissue of *P. ricini* effected by a modern synthetic pyrethroids compound other than used by previous workers. The larvae of *P. ricini* are serious pest of variety of agricultural crops. Therefore, histopathological knowledge due to the action of insecticides with prove useful to asses the effectiveness of an insecticides to control of this species.

MATERIALS AND METHODS

MATERIALS AND METHODS

(i)BREEDING AND STOCK CULTURE OF *PERICALLIA RICINI* FABR.

P.ricini were collected during the month of July to September near the tube light, electric bulbs, and murcury lamps in the night from the Agriculture farm situated in the campus of the Aligarh Muslim University Aligarh, and reared in the laboratory. Several pairs of adults *P. ricini* were released in a circular rearing glass jars measuring 12"x8" and 8"x1". The bottom of each jar was filled with moist sand to a depth of one and a half inch. The top of the circular jar was covered tightly with muslin cloth by means of a rubber band. The culture was maintained in the laboratory at temperature of 28+2°C and 70+5 R.H. and 12:12 hours photoperiod in the B.O.D. incubator. The adults were fed on saturated 5% glucose solution with the help of microslide covered over by sterilized cotton wool which was soacked with the fresh sugar solution. Such slide was prepared for every day feed. For the collection of egg masses a pair of unglazed paper was hanged inside the

rearing jar. It is a good place for resting of the insects, secondly, it serves a suitable place for deposition of egg. Every morning the food was changed. 1st and 2nd instar darvae were fed on tender caster (*Ricinus communis*) leaves. The food was supplied twice a day and the hygienic conditions were maintained by cleaning the jar daily. To avoid over crowding the number of larvae per jar was restricted to 100-150 in Ist instar 50-100 in 2nd instar, 50-75 3rd instar, 20-30 in IVth instar and 10-20 larvae in Vth - VII instar. The leaves were washed under running water every day. Old paper strips and excreta were removed, and fresh strips were introduced. The dead months were also removed and jar was cleaned. After undergoing a series of moulting the larvae pupated and moths emerge. These were transfered to other jars and kept under controlled conditions for mass stock culture, VIth and VIIth instar larvae were sorted out and kept in separate breeding jars.

Egg laying took place after 12-16 hours following copulation and egg hatched after 2-3 days. Generally there are six larval instars and larval period extends upto two weeks. The emergence of adults took place after a week following pupation. For the present studies

healthy IVth instar larvae of lamost some egg were soted out from the culture.

(ii)PREPARATION OF DIFFERENT CONCENTRATIONS OF SYNTHETIC PYRETHROIDS :

Prepared six doses 100 ml each of Deltamethrin 2.8% E.C. as Decis (Hoechst India Ltd.) and Relothrin 25% E.C. (Rellis India Ltd.). Deltamethrin; 0.001%, 0.0025%, 0.005% and Relothrin; 0.001%, 0.0025%, 0.005% were prepared in distilled water through Pearsons square mehtod (Srivastava, 1991).

(iii) APPLICATIONS OF INSECTICIDES :

Each concentation was tested on IVth instar larvae of *P.ricini* separately by dipping the caster leaves with the formulation. These leaves kept for some time in room to dry insecticide. After insecticides get drived the leaves were placed in each jar for feeding. The individuals treated with each concentration were kept in separate jars and maintained at 70+5.RH. The 50% mortality was recorded at more than 0.009% concentraiton of the insecticide during preliminary study. Therefore, for furtherr histopathological studies, the insecticide concentration were kept well below

the above concentration by dilution. The volume of each dilution remained the same.

The preliminary data gave the idea that even with 0.01% concentration 20% larvae died within 24hours. For histopathlogical observation, the tissue of live and treated larvae were studied upto maximum period of 72 hours at regular intervals of 24 hours after the applciaiton of the treated leaves.

(iv) HISTOPATHOLOGICAL PREPARATION :

After the application of each concentration of the insecticide, the larvae were dissected after 72 hours The transected portions of midgut was transferred in aqueous Bouin's fluid for twelve hours. Other fixatives like Carnoys, Zenkers and Alcoholic Bouin were also used but the most satisfactory results were obtained by using aqueous Bouin Solution (**David, 1991**). After twelve hours the material was washed several time in water for about 12 hours so as to remove extra fixative. Each tissue alcohal grades for 30-40 minutes each. Each tissue was then transferred in a mixture of equal quantity of Xylol and 1:10 of mixed Bees wax + Parffin 'Qualialigens) 58-60°C melting point.

The wax embedded materrial were sectioned with the help of Rocking microtome and section were cut at 4-5 microns. The ribbons fixed on a slide heated in oven on stretching plate, until the ribbon was uniformaly strelched. The slides were kept in thermostate at 40+2°C over night for complete evaporation. The slide were placed in Xylol to remove the wax from the sections and than hydrated in series of alchol grades till they were in distilled water and were dehydrated and differentiated through 96% alchol. The material was strained in Heiden hains Iron haemotoxylin and counter strained in alcholic Eosin. After passing through absolute alcohol, they were cleaned in Xylol and finally mounted in D.P.X. for microscopic examination.

Photomicrographs were taken at 4x10 and 10x45 magnification on black and white film for complete gut system, the drawing was made using comeralucida and Praktica camera.

RESULTS

RESULTS

(i) EXTERNAL ANATOMY OF THE NORMAL DIGESTIVE TRACT OF IVTH INSTAR LARVA OF *PERICALLIA RICINI* F. (Fig.1)

The alimentary canal(AC) of IVth instar larva of *P. ricini* is a straight tube with convolusions in certain region. It is morphologicaly divisible into three regions; the foregut or stomodaeum(stom), the midgut or mesenteron (Mesn) and the hindgut or protodaeum (Prot.).

The true mouth is located high up in the head prceeded by the sucking pump. The sucking pump is purely eibaral in region. The stomodaeum and protodaeum are short whereas mesenteron occupies the bulk of the alimentary canal. The stomodaeum is much stortar than the mesenteron and extends upto the metathorax. It is subdivided into a short cylindrical phayrnx, just behind the mouth cavity (cibarium) which is provided with dilator muscles. The pharynx opens into a short oesophages (Oe) which gradually dilates to form a very short crop (cr). The latter is a sac-like structure and its posterior end invagination into the

mesentron to form the cardiac value between the fore and midgut. However a proventricular region is not differentiated.

The midgut is very long but straight tube of approximately uniform dimeter. There are no gastic caecae and the end of the midgut is externally marked by the presence of Malpighian tubules (Mal).

The proctodaeum is much shorter than the midgut. It is constricted at its anterior end to form a short pylorus, which controls the passage of food from the mesenteron to the anterior intestine (ALnt). The anterior intestine is a shorter Sac-shaped like a spindle. The posterior intestine (PLnt) is dilated to form a very small sac known as rectum (Rect) which opens outside through the anus.

Malpighian tubules which are yellowish in colour are long and hihgly convaluted tube which hang freely in the posterior abdominal cavity. These tubules enclose the outer wall of the rectum in a convoluted manner to make cryptonephridial arrangement. The entire alimantary canal is covered with fine trachea which are round in shape and cover the entire periphery of alimentary canal.

(ii) EXTERNAL ANATOMY OF THE TREATED DIGESTIVE TRACT OF THE 4th INSTAR LARVA OF THE *P. RICINI* F. (Fig. 2&3)

Deltamethrin treated digestive tract of *P. ricini* larvae showed considerable shrinkage of the midgut region at all the concentrations used. However, at lower concentrations the shrinkage was less pronounced. The malpighian tubules were found to be disturbed at certain points, also sharing the effect of insecticide.

Treatment of relothrin caused comparatively less pronounced effect in external anatomy of the digestive tract of *P. ricini* larvae gut, especialy midgut region which shrunk a little longwith a slight distribance in the position of malpighian tubules.

(iii) HISTOLOGY OF MIDGUT OF 4TH INSTAR LARVA OF *PERICALLIA RICINI* F (Plate-I, Fig. A&B)

The midgut wall of the 4th instar larva of *P. ricini* is bound by a muscularis which is composed of an outer layer of longitudinal muscle fibres and an inner layer of circular muscle fibre (Conl). The longitudinal muscle bundles are uniformly spaced around the midgut wall Each muscle bundle is composed of 4-6 bands which are closely packed. The circular layer is a compact layer of fibres running aroun the inner connective tissue. Circular muscle fibres are internally lined with a structureless membrane called basement membrane. The inner margin of the connective tissue is the basement membrane (BMb) which support the epithelium of the midgut. The midgut epithelium is compressed when midgut is fully packed with the food. However, in the partially filled up midgut condition the epithelium is relaxed the epithelium is composed of a single layer of three types of cell as given below.

(a) Columner cell (Ccl):

These cells are tall and have a centrally placed muscles The cytoplasm of these cells is highly granular and eosinophilic. The nucleus of each cell in oval

and contains fire granules which are uniformally scattered in the nuclear (Nu) space The nuclear content is deeply basophilic the inner margin of these cells is provided with a brush like striated border (sb) These cells constitute the bulk of the epithelium.

(b) Goblet cell (G cl):

These cells are scattered among the columner cells and may be found in groups of 2 - 3 cells. Each goblet cell has scanty cytoplasm. The inner margin of the goblet cells which bear striated border is invaginated deep into the cell. Therefore, nucleus comes to lie in the basal region of the cell and becomes discoidal. The opening of the goblet cells in the midgut lumen is narrow and lined with the straited border. The cytoplasm is less granular and weakly eosinophile. In the anterior region of midgut distribution of the goblet cells is more sparse than in the middle and posterior regions. These cells are more numerous and lie in group of 2-3 after one or two celumner cells. Further in these regions the goblet cells appear to be flask like. Both columnar and gablet cells are the digestive cells of the epithelium.

(c) Regenerative cells (RgC):

These are small cells lie in groups of 5-6 near the baement memberane in relation to the columner cells. Each group of these cells or nidus (Ni) is distributed irreglarly and sometimes regularly throughout the epithelium. Each cell of the nidus is very small with scanty eosinophilic eytoplasm and invisible cell membran but their nuclei are irregular, strong bosophilic and have granular contents.

The inner most layer of the midgut is peritrophic membran (Pmb.) which is protective in nature. This layer lines close to the epithelium, irregular arrangement and is without any definite cell structure.

The innermost space of the midgut is lumen (lm) whose diameter is narrow in the anterior region of the midgut and gradually increases in the posterior region.

(iv) HISTOPATHLOGY OF MIDGUT OF FOURTH INSTAR LARVAE OF *PERICALLIA RICINI* F TREATED WITH DIFFERENT CONCENTRAION OF DELTAMETHRIN AND RELOTHRIN :

The important changes in the histology of midgut of fourth instar larvae of *P. ricini* F. which are treated with different concentration of Deltamethrin and Relothrin is given below-

A. EFECT OF DELTAMETHRIN OF MIDGUT

(a) Effect of 0.001% Deltamethrin (Plate-II, Fig. A&B):

A number of changes occured after 72hr. of the treatment of 0.001% Deltametrin on midgut of the larva of *P. ricini*. Histopathologically some changes were observed in the treated midgut epithlium section as compared to the control sections. 0.001% Deltamethrin treated midgut epithelium showed considerable shrinkage in size. There is a considerable reduction in the size of longitudinal and circular muscle fibre with no clear basement membrane. The size of gut epithelium is also reduced. The peritrophic membrane is seen broken at certain palces. Consequent to the reduction in the size if the cellular part of gut, the lumen becomes greatly enlarge. Certain areas of midgut epitherlium become stretched a little inwords.

(b) Effect of 0.0025% Deltamethrin (Plate III Fig. A&B):

Application of this concentration showed similar alteraitons in the histology of *P. ricini* midgut after 72 hours. Consequent to the effect of treatment the gut losses its circular shape. which is mainly due to damage in the circular and longitudinal muscle fibres. The size of longitudinal muscle fibre bundles are greatly reduced in the layer of circular muscle fibres. Epithelium of anterior and middle region of midgut was found to stretch inwards. The cytoplasm material of the digestive cell aggregated at certain places. Cytoplasmic granules and vesides were secreted through the striated border. Nuclei of digestive cells were damaged . The connective tissue developed vocules. there was dischage of cytoplasmic granules in the obliterated strailed borders.

c. Effect of 0.005% Deltmethrin (Plate lV Fig. A&B):

This concentration of the insecticide caused considerable shrinkage in the midgut of the larva of *P. ricini*. Stretching of the anterior epithelium of the midgut was also observed. The treated midgut looses its shape considerably. The bundles of longitudinal muscle fibre and circular muscle fibre layer are greatly reduced. Due to

damage in the muscle fibres the two opposite walls of midgut comes close to each other resulting in reduction in the size of lumen. Straited border become obliterated. The connective tissue get degenerated and destruction could be observed and alteration in the shape of nuclei was also apparent from the sections. The peritrophic membrane is considerably damages ad is broken at many places.

B. EFFECT OF RELOTHRIN ON MIDGUT

(a) Effect of 0.001% Relothrin (Plate, V, Fig. A&B):

Application of Relothrin at a concentration of 0.001% caused little shrinkage in the midgut of larva of *P. ricini*. The size of longitudinal muscle fibre bundle and ciruclar muscle fibre layer is considerably reduced. Epithelium detached from the muscular layer at certain points. Digestive cells developed vacules and pressed scanty cytoplasmic material in the outer regions. The border of striated layer become indistict. No significant change was observed in the other cellular structures. The peritrophic membrane is considerably shrunken and broken leaving the gut epithelium further damage

(b) Effect of 0.0025% Relothrin (Plate VI, Fig. A&B):

This concentraion also caused changes and alteraions like the lower concentration of this insecticide. The longitudianl muscle fibre bundle is reduced and shrunk at certain places in the midgut. The circular muscles fibre layer is also shruck with no distinct basement membrane. Midgut shrunk mover as compaired to the control midgut. Connective tissue ruptured at some places and vacules developed. The nuclei of the cells lost their original

shape. Striated border obliterated at many places and were found to shed in the lumen. The nuclear granules changed giving dark coloured stain. Detachment of the epitherlium was also observed in some parts of midgut. The peritrophic membrane is also damaged and broken at certain places. The main damage of this membrane while reduction in the size of cellular part of the midgut is attributed to the reducton in the size of larva.

(c) Effect of 0.005% Relothrin (Plate VII, Fig. A&B):

The histopathological changes brought about by this concentraion of relothrin caused higher intensity of alteration in midgut. General shape of the midgut of treated larval midgut showed a marked shrinkage inward stretching and vacuolization at some parts. The longitudinal muscles fibre bandle moves to lie at one place and is also reduced in size. The layer of circular muscle fibre are also reduced. The damage of epithelium and digestive cells was clearly apparent. Nuceli of the digestive cells migrated towards the periphery of the cells and lose their normal shape. The nuclear material of the altered nucleii formed a dark stain mass. Connective tissue was found to be highly degenerated with vacules at many

palces. The peritropic membrane is also damaged and seen broken at many places. It is also shrunken and distingrated in the middle if the lumen. The size of lumen is also reduced.

DISCUSSION

DISCUSSION

Application of different concentration of the synthetic pyrethroids (Deltamethrin and Relothrin) showed considerable alterations in the morphology of the alimentary canal. Shrinkage of the larval gut was observed with all the doses of both insecticides when compared to its length in the untreated 4th instar larvae. Deltamethrin was found to cause more distinct shrinkage in the larval gut, particularly at the midgut region where a little bending was also noted in the Delthamethrin treated larvae. The shrinkage in the larval gut after the treatments is perhaps due to the alteration of epithelial tissue and muscular fibres which shrunk and get damaged resulting in the lengthwise shortening of the gut. Malpighian tubules also shrunk and undergone colour change. General shrinkage of all the affected tissues was noticed in the study of histological changes in the tissue of insect with the applciaiton of a number of insecticides. The damage caused to midgut by 0.001% deltamethrin was less pronounced

althrough there were some symptoms of damage in epithelial cell, showing reduction in size. Besides this, reduction in the size of longitudinal and circular muscle fibres was also noted. Broken peritrophic membrane and enlargement of the lumen of gut are other effects of insecticidal treatment. **Lakshmanlal et al;** (1970) has obtained similar results for the midgut epithelium of *Spodoptera litura* and *Periplaneta americana* respectively using organochlorin insecticides. Epithelium was also found to be stretched inwards at some points. Several other authors have studied the effects of insecticides on midgut epithelium of different insects. (**Pilat**, 1935; **Salkeld**, 1951; **Soliman** & **Soliman** 1958 and **Farooqui**, 1972).

The damage caused by the 0.001% relothrin was comparatively less pronounced, though the epithelium appeared to detatch at some points while size of longitudinal muscle bundles and muscles fibre layer was also reduced. The epithelium of midgut of a number of insect has been found to be affected drastically by different insecticides as reported by **Srivastava** (1962), **Rizvi** and **Khan** (1973), **Shukla et al;** (1977) and **Sabesan** and **Rainlingam** (1978).

The second higher concentration 0.0025% of all deltamethrin also damaged circular and longitudinal muscle fibres as a result of which the gut loses its circular shape. This higher dose caused similar kind damage to the epithelium and cillular material but the severety of the damage was comparatively more pronounced.

Changes brought about by 0.0025% relothrin were also more as less similar as that caused by lower concentration of the insecticide except the development of vacuoles and damage connective tissue. Since the vocualization was also observed in the tissues of the midgut of *P. ricini* F. larva, it can be presumed that shrinkage is caused by the expulsion of water from the tissue when treated with insecticides.

Deltamethrin at a higher concentration of 0.005% was most destructive for the midgut of the larva. Damage was observed in all the important tissue like epithelium longitudinal and circular muscles and cellular structures, besides the peritrophic membrane. The histopathological effects of relothrin at 0.005% concentraiotn also caused serve alteration but comparatively less than that caused by this concentration of the former

insecticides. The destruction was apparent in connective tissue, epithelium, circular and longitudinal muscle fibre. Peritrophic membrane was also damaged at this concentration.

The damage to the peritrophic membrane is pronounced among all tissues of midgut at various concentration of two insecticides. This is due to the fact that the different concentraion of this insecticides damage the peritrophic membrane first . The rupture of peritrophic membrane at different point made the tissue of midgut ware vulnevable to insecticide action as protective covering of epithelial tissue is lost.

A comparative data, thus reveal that the midgut epithelium is the most sensitive and susceptible part of the midgut of the toxic effects of both the synthetic pyrethruds. If we observe the comparative damage of muscular layer it will be very clear that epithelium is more effective part and it is due to the fact that epithelial layer of the midgut is more frugile than the muscularis. At higher concentrations of pyrethroide insecticides are found to cause detachment of the gut epithelium from the basement membrane. Insecticides act upon the cellular contents of the epithelial cells and bring about abnormalities like changes

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is shape nucleus chumping etc. Similar but more serve damage of midgut epithelium and cellular contents is reported in grosshopper *Chrotoganus praenypeterus* caused by ethyle parathion Singh, (1990).

In the study on the effects of some ingested insecticides on the midgut wall of the VIth instar of Southern armywarm larva by Woke (1940). The study shows that different insecticides has different action from no apparent effect to well marked damage to the midgut wall.

It is quite clear from the above discussion that both the pyrethroid insecticides, is deltamethrin and relothrin at lowers as well higher concentraion caused damage to the midgut of the IVthe instar larvae of *P. rincini* Epithelial tissue and peritrophic membrane is more damage besie the crcular and longitudianl muscles fibres also get damage at higher concentrations.

Cellular contents and basement membrane also get altered at higher concentraion In generral the effect of the two insecticides used so far, show marked damage to the midgut, However, as compared to the effects. Other insecticides on different insects, the synthetic pyrethroida are less effective in bringing about histopalogical

attraction in midgut tissue. This pyrethroids being contact poison, show low damage. As for the relative effects of the two pyrethroid insecticides on the *P. ricini* midgut, it can be said that the deltamethrin is comparatively more toxic that that of the relothrin. The farmer insecticide caused more damage to the midgut even at lower concentrations.

SUMMARY

SUMMARY

It has been reported that synthetic prethroids have extremely high contact activity and are particularly effective against larvae of insects. They are effective at low doses and have low mammalian toxicity. Therefore, favourable toxicalogical and ecological properties instigate the widespread application of these sysnthetic pyrethroids in controlling posts counting damage to the agricultural crops and products.

Present study has been undertaken to have an idea as to whether such pyretheroids when taken into the alimentary canal cause any adverse effect on the gut lining. Two synthetic pyrethroids, Deltamethrian and Rolothrin. thus, were chosen and IVth instar larvae of *Pericallice ricini* F. were fed pyrethroid insecticides treated foliage. The general anatomy of alimentary canal, histology of midgut and histopathology of midgut was studied and compared with that of the gut of untreated larvae.

Results showed damage of the midgut epithelium, circular and longitudinal muscle fibre peritrophic and basement membranes alongwith the considerable alterations in the cellular structures. The damage to the peritrophic membrane is more pronounced at different concentration of insecticides.

The higher concentration of the insecticides caused more histopathological abnornamalities in the midgut of treated larvae. Moreover, the deltamethrin was found to be caused more damage than the relothrin an the midgut of 4th instar larva of *P.ricini*. The part of the midgut most affected was peritrophic membrane, Further, the cell structure were also got damaged by the application of the pyrethroid insecticides.

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* Not seen in Original

FIGURES AND PLATES

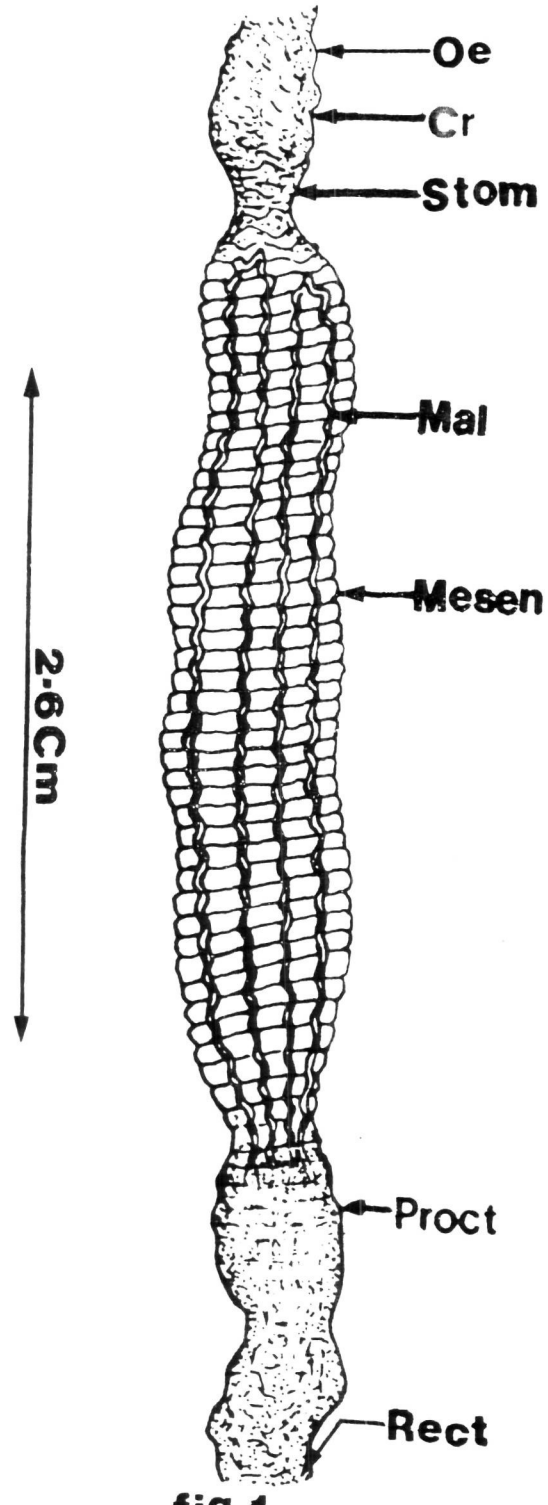
(FIGURES)

Fig.1. External anatomy of normal digestive tract of 4th instar larva of P. ricini. F.

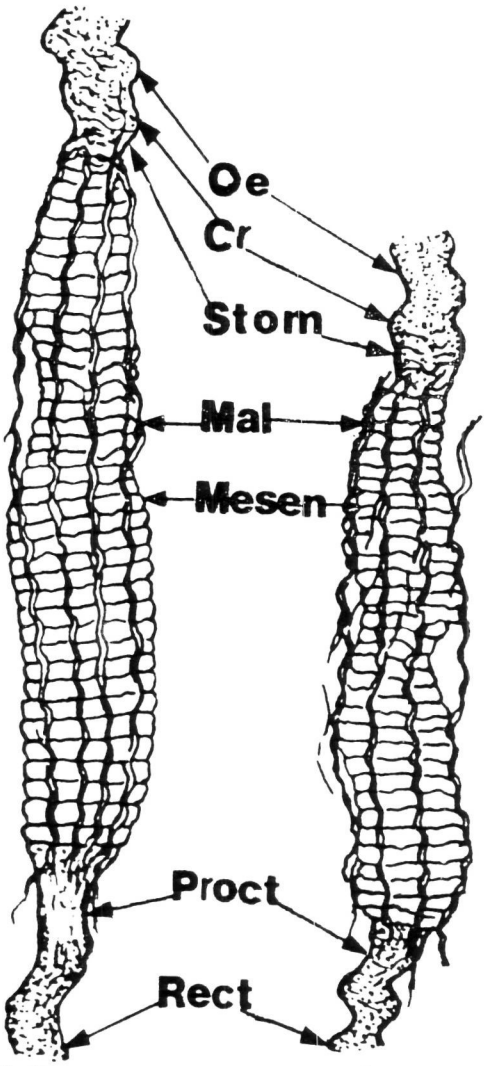
Fig.2. External anatomy of disestive tract of 4th instar larva of P. ricini.F. treated by Relothrin.

Fig.3. External anatomy of digestive tract of 4th instar larva of P. ricini.F. treated by Deltamethrin.

Figures



fig·1



fig·2

fig·3

(PLATE - I)

Fig. A&B. T.S. of normal midgut of fourth instar larva of P. ricini.F.

PLATE - I

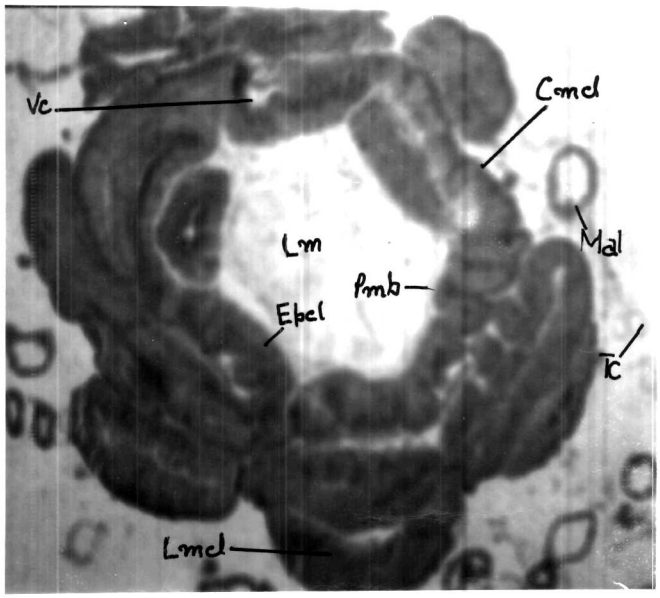


Fig. A (4 x 10 mag.)

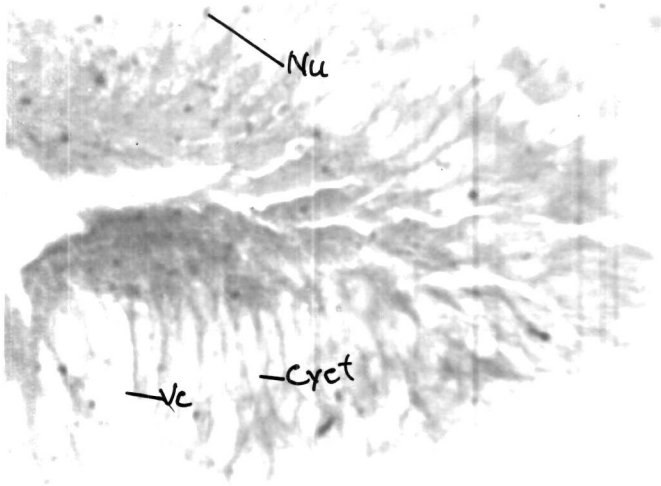


Fig. B (10 x 45 mag.)

(PLATE- II)

Fig. A&B-T.S. of midgut of fourth instar larva of P. ricini. F. treated by 0.001% of Deltamethrin.

PLATE - II

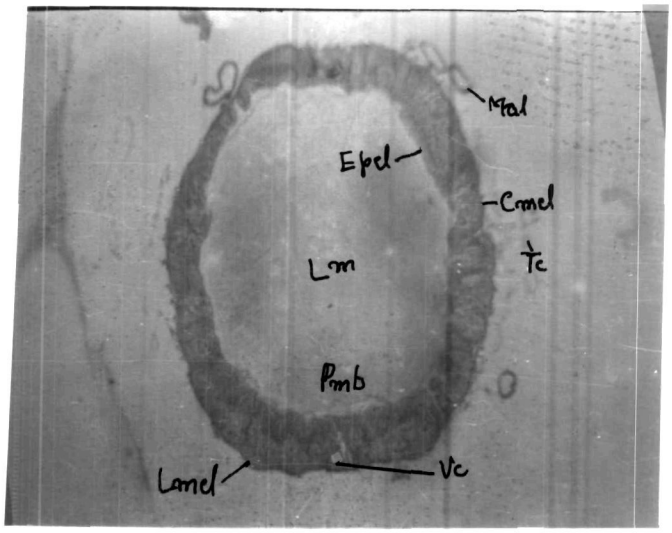


Fig. A (10 x 4 mag.)

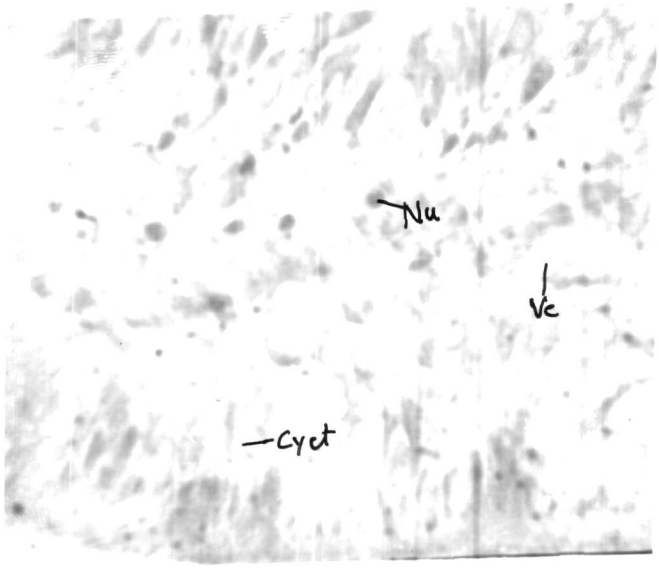


Fig. B (10 x 45 mag.)

(PLATE- III)

Fig. A&B- T.S. midgut of fourth instar larva of P. ricini. F. treated by 0.0025% of Deltamethrin.

PLATE - III

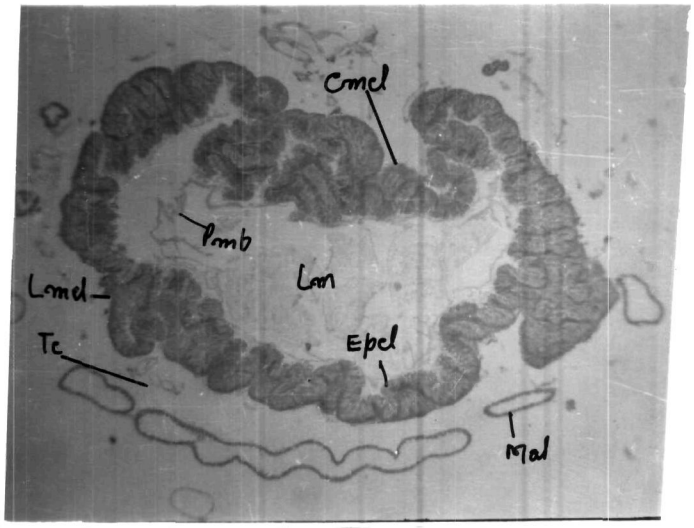


Fig. A (10 × 4 mag.)

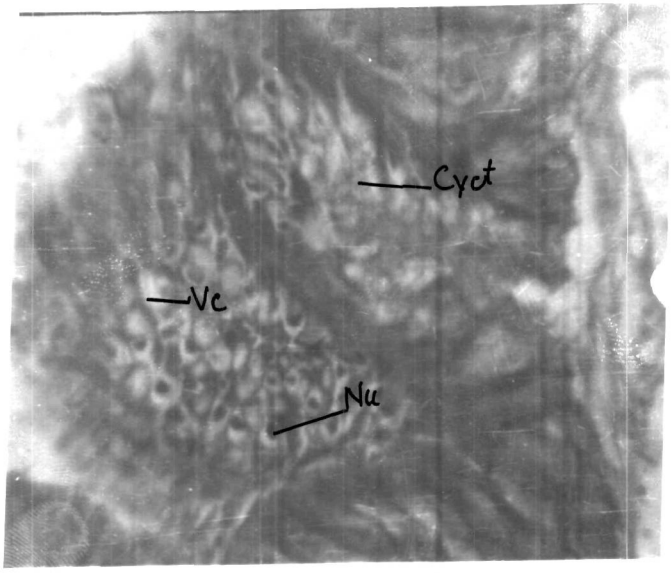


Fig. B (10 × 45 mag.)

(PLATE - IV)

Fig. A&B-T.S. of midgut of fourth instar larva of P. ricini. F. treated by 0.005% of Deltamethrin.

PLATE - IV

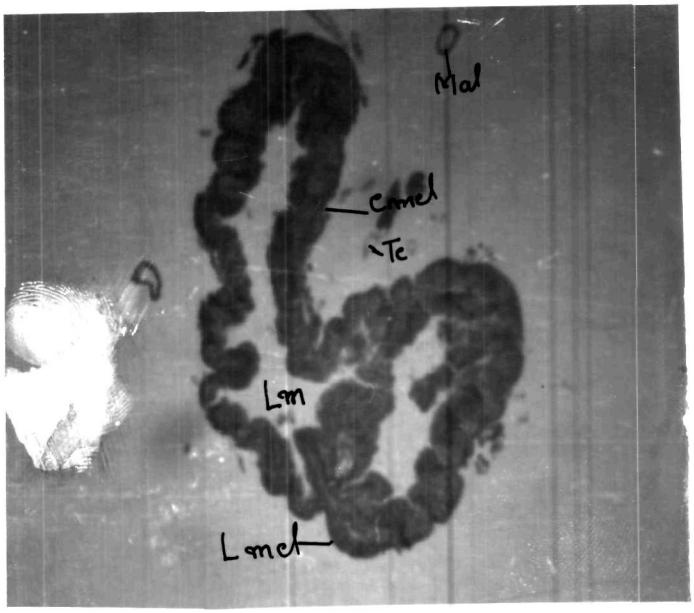


Fig. A (10 × 4 mag.)

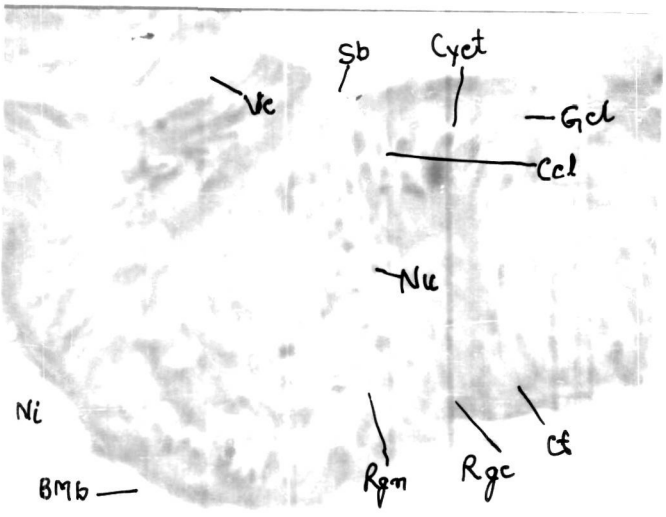


Fig. B (10 × 45 mag.)

(PLATE- V)

Fig. A&B- T.S. of midgut of fourth instar larva of *P. ricini*. F. treated by 0.001% of Relothrin.

PLATE - V

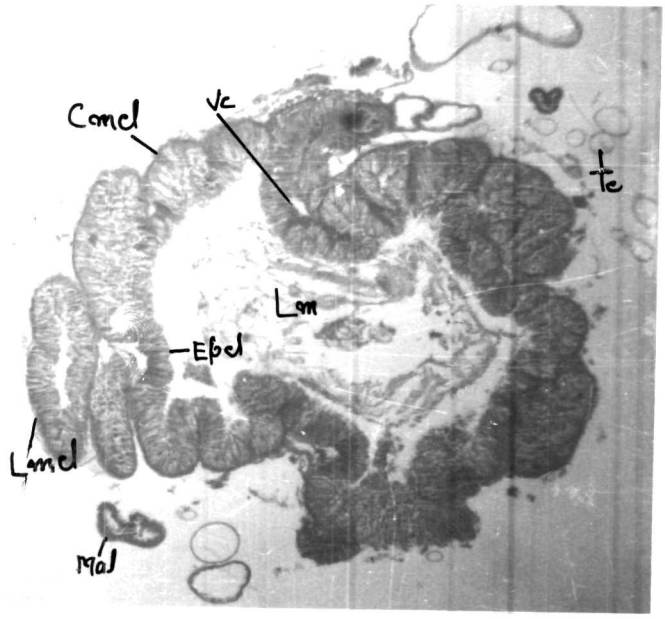


Fig. A (10 X 4 mag.)

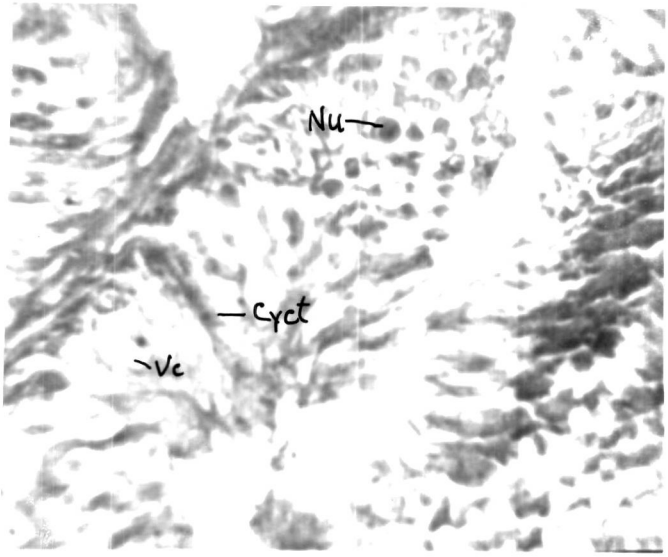


Fig. B (10X 45 mag.)

(PLATE- VI)

Fig. A&B-T.S. of midgut of fourth instar larva of P. ricini. F. treated by 0.0025% of Relothrin.

PLATE - VI

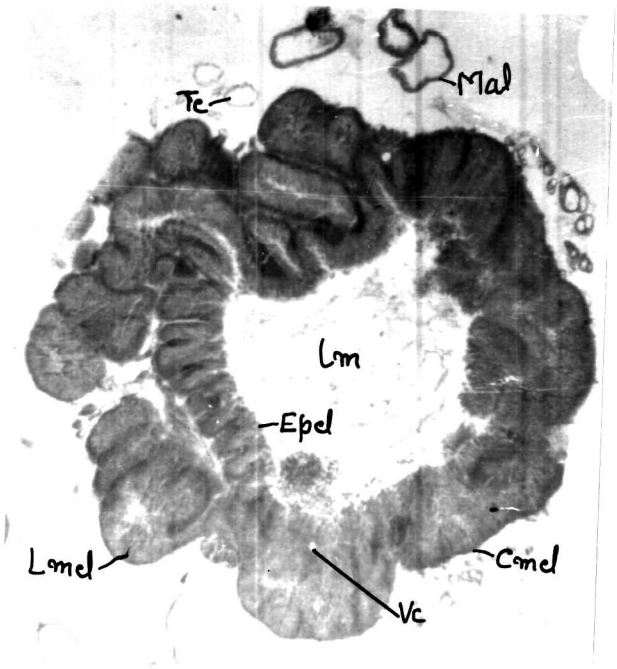


Fig. A (10 x 4 mag.)

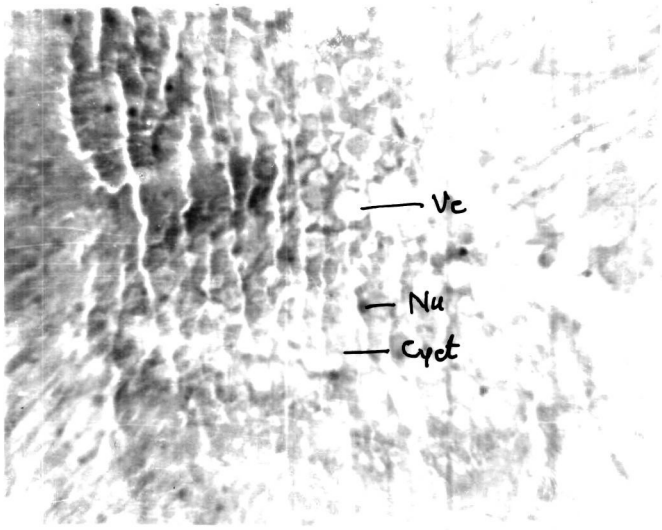


Fig. B (10 x 45 mag.)

(PLATE- VII)

Fig. A&B-T.S. of midgut of fourth instar larva of P. ricini. F. treated by 0.005% of Relothrin.

PLATE - VII

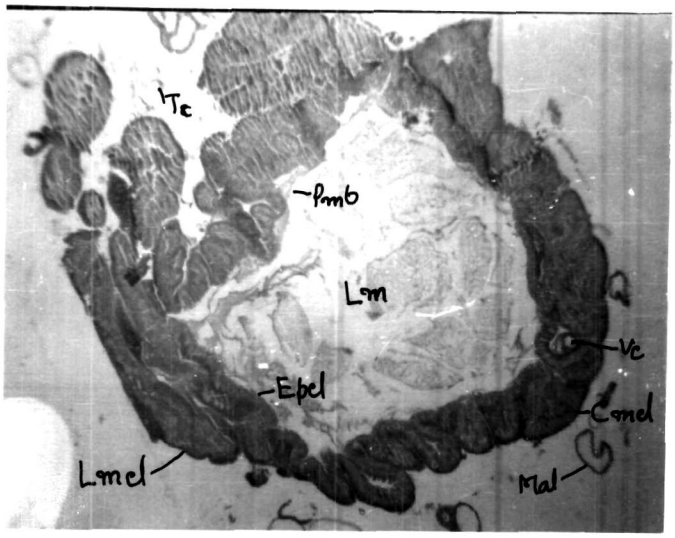


Fig. A (10 X 4 mag.)

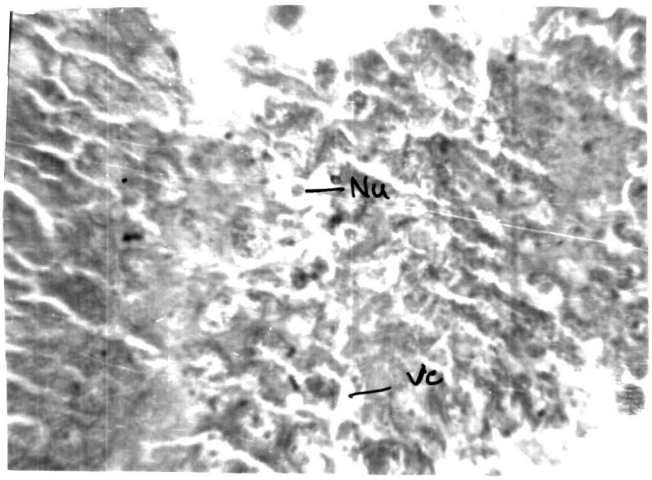


Fig. B (10 X 45 mag.)